Callicladol, a Novel Cytotoxic Bromotriterpene Polyether from a Vietnamese Species of the Red Algal Genus *Laurencia*

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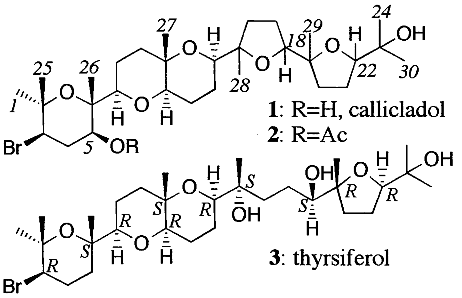
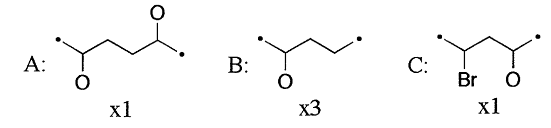
Callicladol, a novel brominated metabolite has been isolated from *Laurencia calliclada* Masuda sp. ined., a Vietnamese species of the red algal genus *Laurencia.* Its structure was deduced from spectral and chemical evidence.

To date there has been no report concerning the chemical study of specimens of the red algal genus *Laurencia* found in Vietnamese waters. In connection with our chemotaxonomic studies of the genus *Laurencia*, we collected *Laurencia* species in several locations in Vietnamese waters. One of them, *Laurencia calliclada* Masuda sp. ined. collected at An Thoi, Phu Quoc Island, Kien Giang Province, on February 8, 1993, was found to contain a novel brominated triterpene polyether, designated callicladol, as the characteristic metabolite of this species. In this paper, we wish to report the structural elucidation of this unique bromotriterpenoid with a pentacyclic skeleton.



A combination of column and thin-layer chromatography of methanol extracts has led to the isolation of callicladol (**1**) as needles in 6.4% yield based on the extracts. Callicladol (**1**), mp 198-199°C (EtOH), $[\alpha]_D^{23}$ +75.1° (*c* 0.60, $CHCl_3$), displayed a cytotoxic activity *in vitro* against P388 murine leukemia cell with IC_{50} of 1.75 μg/ml, and its molecular formula was analyzed for $C_{30}H_{51}BrO_7$ by HR-FABMS.[1] Treatment of **1** with acetic anhydride and pyridine gave the corresponding monoacetate **2**,[2] $C_{32}H_{53}BrO_8$, ν_{max} 1722 cm^{-1} and δ_H 1.95 (3H, s), whose IR spectrum still showed an absorption due to hydroxyl group at 3384 cm^{-1}, thus proving the presence of secondary and tertiary hydroxyl groups in **1**. The ^{1}H and ^{13}C NMR spectral data revealed that callicladol (**1**) possessed 8 tertiary methyls, 6 oxygenated quaternary carbons, 5 oxygenated tertiary carbons, and a brominated tertiary carbon (δ 54.24). Since callicladol (**1**) had not any unsaturated bond, **1** was assumed to comprise five oxide rings. The detailed analysis of the ^{1}H-^{1}H COSY, HOHAHA, and HSQC spectra of **1** showed the presence of the same partial structural units A and B (Figure 1) in the molecule as those in thyrsiferol (**3**) isolated from *Laurencia thyrsifera*.[3] Moreover, a unit C was present in **1** instead of a 1-bromotrimethylene moiety which commonly presents in thyrsiferol (**3**) and its congeners, e.g. venustatriol,[4] magireols,[5] and 15-anhydrothyrsiferyl derivatives.[5]



(• : Quaternary carbon atom)

Figure 1. Partial structural units for callicladol (**1**).

The ^{1}H-^{13}C long range correlations from the HMBC spectrum of **1** could not lead to the gross structure for **1** because only 27 carbons were detected in its ^{13}C NMR spectrum. However, in ^{13}C NMR spectrum of the acetate **2**, 30 carbons were detected together with two overlapping carbons, and partial HMBC correlations were observed as illustrated in Figure 2.

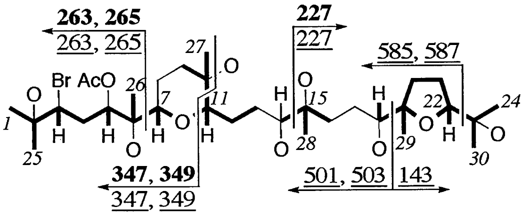


Figure 2. HMBC correlations (—), and the fragment ions (*m/z*) in EI (underline) and FAB (**bold**) mass spectra of monoacetate **2**.

The ring system was deduced from the specific fragment ions in EI and FAB mass spectra (Figure 2). The mass spectra of **2** showed the fragment ion at *m/z* 143 $[M-C_{24}H_{38}BrO_6]^+$ due to cleavage at C18-C19 bond. The presence of an additional oxolane ring arising from ether bridge between C15 and C18 was evident from the fragment ion at *m/z* 227 $[M-C_{19}H_{30}BrO_5]^+$. Furthermore, the fragment ions at *m/z* 263, 265 $[M-C_{22}H_{37}O_5]^+$ indicated the presence of a 2-acetoxy-4-bromo-1,5-dimethyl-1,5-epoxyhexyl group, and hence the remaining two ether linkages consisted of 2,7-dioxabicyclo[4.4.0]decane ring. Consequently, callicladol (**1**) was found to be a new member of squalene-derived bromotriterpene belonging to congeners of thyrsiferol (**3**). The equatorial nature of both substituents of the bromine atom at C3 and the hydroxyl group at C5 on the A ring was evident from the *J*-values with vicinal axial-axial coupling constants of H-3 (12.4 Hz) and H-5 (11.4 Hz) in the ^{1}H NMR spectrum of **1**.

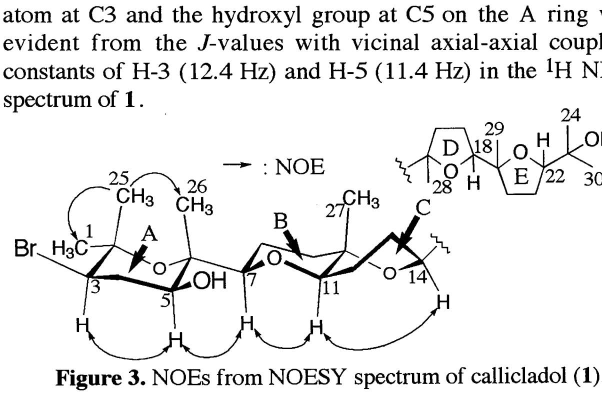


Figure 3. NOEs from NOESY spectrum of callicladol (**1**).

Table 1. ^{13}C(100 MHz) and ^{1}H(400 MHz) NMR data (in $CDCl_3$) for callicladol (**1**)

Pos.	^{13}C δ		^{1}H δ (*J* in Hz)
1	30.32 or 30.36	q	1.26 (*s*)
2	75.42	s	
3	54.24	d	3.86 (*dd*), *J*=12.4, 4.1
4	35.11	t	2.20 (*m*) and 2.30 (*m*)
5	76.41	d	3.64 (*dd*), *J*=11.4, 4.2
6	76.41	s	
7	88.95	d	3.25 (*dd*), *J*=11.2, 2.4
8	22.66	t	1.50 (*m*) and 1.73 (*m*)
9	38.30	t	1.50 (*m*) and 1.78 (*m*)
10	71.09	s	
11	76.41	d	3.64 (*dd*), *J*=11.2, 7.3
12	21.25	t	1.55 (*m*) and 1.95 (*m*)
13	20.61	t	1.80 (*m*) and 1.95 (*m*)
14	75.33	d	3.73 (*dd*), *J*=12.4, 3.2
15	84.46	s	
16	33.16	t	1.52 (*m*) and 2.27 (*m*)
17	28.79	t	1.49 (*m*) and 1.87 (*m*)
18	86.27	d	4.09 (*dd*), *J*=10.3, 5.4
19	85.74	s	
20	30.36 or 30.32	t	1.47 (*m*) and 2.17 (*m*)
21	26.24	t	1.99 (*m*) and 2.13 (*m*)
22	85.74	d	3.85 (*dd*), *J*=8.1, 3.7
23	72.20	s	
24	27.85	q	1.24 (*s*)
25	23.01	q	1.38 (*s*)
26	15.11	q	1.22 (*s*)
27	21.36	q	1.20 (*s*)
28	24.76	q	1.11 (*s*)
29	25.07	q	1.12 (*s*)
30	25.24	q	1.06 (*s*)

Table 2. ^{13}C(100 MHz) and ^{1}H(400 MHz) NMR, and HMBC data (in $CDCl_3$) for monoacetate **2**

Pos.	^{13}C δ		^{1}H δ (*J* in Hz)	Long range correlations
1	30.31	q	1.28 (*s*)	H_3-25, H-3
2	75.31	s		H_3-1, H_3-25, H_2-4
3	53.29	d	3.88 (*dd*), *J*=12.4, 4.7	H_3-1, H_3-25, H_2-4
4	34.04	t	2.23 (*m*) and 2.28 (*m*)	H-3, H-5
5	73.81	d	4.96 (*dd*), *J*=11.2, 5.4	H_3-26, H_2-4, H-7
6	75.99	s		H_3-26, H_2-4, H-7
7	86.60	d	3.14 (*dd*), *J*=11.2, 2.4	H_3-26, H-5, H-11
8	23.06	t	1.40 (*m*) and 1.70 (*m*)	
9	38.64	t	1.46 (*m*) and 1.72 (*m*)	H_3-27, H-11
10	71.09	s		H_3-27, H-11
11	76.59	d	3.44 (*dd*), *J*=10.7, 7.3	H_3-27, H-7
12	21.05	t	1.41 (*m*) and 1.82 (*m*)	H-11
13	20.59	t	1.74 (*m*) and 1.91 (*m*)	H-11
14	75.13	d	3.69 (*dd*), *J*=12.7, 2.5	H_3-28
15	84.49	s		H_3-28
16	33.25	t	1.49 (*m*) and 2.28 (*m*)	H_3-28
17	28.84	t	1.46 (*m*) and 1.86 (*m*)	
18	86.21	d	4.10 (*dd*), *J*=10.3, 5.4	H_3-29
19	85.75	s		H_3-29, H-22, H-18
20	30.30	t	1.46 (*m*) and 2.17 (*m*)	H_3-29, H-22, H-18
21	26.26	t	1.97 (*m*) and 2.13 (*m*)	
22	85.75	d	3.84 (*dd*), *J*=8.1, 3.2	H_3-24, H_3-30
23	72.20	s		H_3-24, H_3-30
24	27.85	q	1.24 (*s*)	H_3-30
25	22.90	q	1.40 (*s*)	H_3-1
26	15.80	q	1.26 (*s*)	H-5, H-7
27	21.42	q	1.16 (*s*)	H-11
28	24.73	q	1.08 (*s*)	
29	25.11	q	1.12 (*s*)	
30	25.26	q	1.06 (*s*)	H_3-24
Ac	21.42	q	1.95 (*s*)	
Ac	169.8	s		

The relative stereochemistries were determined by the NOESY spectrum as shown in Figure 3. The NOEs about the ABC-ring were detected between H-3/H-5, H-5/H-7, H-7/H-11, and H-11/H-14, respectively, indicating that the all methine protons on the ABC-ring were oriented to the axial direction. These data as well as biogenetic viewpoint strongly suggested that callicladol (**1**) possessed the same ABC-ring system as that of thyrsiferol (**3**) and its congeners. In addition, no NOE between H_3-29/H-22 and H_3-28/H-18 suggested the stereochemistries of the E and D ring to be *trans*. The relative configurations, however, between H-14/H_3-28 and H-18/H_3-29 remained unsettled.

The determination of the absolute configuration of secondary hydroxyl group at C5 by the application of the advanced Mosher's method[6] failed because MTPA ester could not be formed by the steric hindrance of the BC-ring toward the C5-OH.

Callicladol (**1**) is the first example of the halogenated squalene-derived polyether from the genus *Laurencia* with a hydroxyl group at C5.

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References and Notes

1 Callicladol (**1**): IR ($CHCl_3$); ν_{max} 3490, 3396, 2972, 2866, 1456, 1378, 1318, and 948 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); LR-EIMS *m/z*: 604, 602 (0.2:0.1) $[M]^+$, 545, 543 (0.8:0.8) $[M-C_3H_7O]^+$, 461, 459 (1.0:1.4) $[M-C_8H_{15}O_2]^+$, 307, 305 (3.5:3.3) $[M-C_{17}H_{29}O_4]^+$, 277 (100) $[M-C_{17}H_{28}BrO_4]^+$, and 143 (68) $[M-C_{22}H_{36}BrO_5]^+$; LR-FABMS *m/z*: 605, 603 (29:26) $[M+H]^+$, 587, 585 (46:36) $[M-H_2O]^+$, 307, 305 (15:29) $[M-C_{17}H_{29}O_4]^+$, and 227 (74) $[M-C_{17}H_{28}BrO_4]^+$; HR-FABMS *m/z*: 603.2906, Calcd for $C_{30}H_{52}{}^{79}BrO_7$, 603.2890 [M+H].

2 **2**: Colorless oil; $[\alpha]_D^{23}$ +37.4° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$); ν_{max} 3384, 2968, 2866, 1722, 1456, and 1375cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); HR-FABMS *m/z*: 645.2976, Calcd for $C_{32}H_{54}{}^{79}BrO_8$, 645.3002 [M+H].

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